Carbohydrate Research, 92 (1981) 65–74
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

β-ELIMINATION OF ALDOBIOURONIC ACIDS DURING METHYLATION BY THE HAKOMORI METHOD*

KAZUMASA SHIMIZU

Forestry and Forest Products Research Institute, P.O. Box 16, Tsukuba Norin Kenkyu Danchi-nai, Ibaraki 305 (Japan)

(Received November 3rd, 1980; accepted for publication, December 1st, 1980)

ABSTRACT

On methanolysis and subsequent saponification, the aldobiouronic acids 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**) and 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose (**2**) gave four (**3–6**) and two (**7** and **8**) methyl glycosides, respectively. These glycosides were well separated by ion-exchange chromatography and their structures were established by methylation analysis, mass spectrometry of their permethylated derivatives, and ^{13}C-n.m.r. spectroscopy. On methylation by the method of Hakomori, compounds **3–6**, having 4-*O*-substituted uronic acid residues, afforded β-elimination products (**15–18**) in addition to the permethylated derivatives (**9–12**). On the other hand, compounds **7** and **8**, having HO-4′ unsubstituted, gave the products of direct methylation (**13** and **14**), which gave, on re-methylation, permethylated, unsaturated aldobiouronic acids (**19** and **20**).

INTRODUCTION

Successful Hakomori methylations of acidic polysaccharides containing 4-*O*-substituted hexuronic acid residues have been reported[1]. However, 4-*O*-methyl-α-D-glucopyranosyluronic acid residues in xylan undergo degradation by β-elimination during such methylation[2,3]. We now report on the methylation of methyl glycosides of the aldobiouronic acids 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**) and 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose (**2**).

RESULTS AND DISCUSSION

Ion-exchange chromatography of the saponified products of the methanolysis of **1** and **2** gave four (**3–6**) and two (**7** and **8**) acid components, respectively. Acid hydrolysis of these products regenerated the parent compounds, indicating that they were methyl glycosides. They were identified on the basis of ^{13}C-n.m.r. spectroscopy, methylation analysis, and e.i.- and c.i.-mass spectrometry of their permethylated

*Presented in part at the 28th Annual Meeting of the Japan Wood Research Society, 1978, Nagoya.

0008-6215/81/0000–0000/$ 02.50,

TABLE I

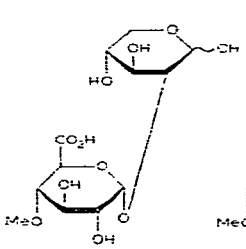
ANALYTICAL DATA FOR METHYL GLYCOSIDES OF ALDOBIOURONIC ACIDS

Methyl glycoside	$[\alpha]_D$ *(degrees)*	D_v *value*		*Equiv. wt.*[c]
		0.02M NaOAc[a]	*0.5M HOAc*[b]	
3 Me 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-α-D-Xyl*f*	+194	5.64	10.83	365
4 Me 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-β-D-Xyl*f*	+78	6.43	12.90	360
5 Me 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-α-D-Xyl*p*	+132	6.36	10.09	350
6 Me 2-*O*-(4-*O*-Me-α-D-GlcA*p*)-β-D-Xyl*p*	+71	7.59	9.21	348
7 Me 4-*O*-(α-D-GalA*p*)-α-D-Xyl*p*	+168	7.93	6.47	330
8 Me 4-*O*-(α-D-GalA*p*)-β-D-Xyl*p*	+64	8.77	6.94	335

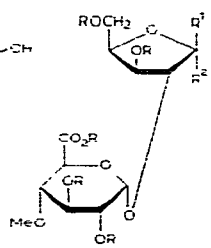
[a]Column (5 × 700 mm) of Diaion resin (acetate form, 23–25 μm). [b]Column (5 × 500 mm) of Aminex A-27 resin (acetate form, 12–15 μm). [c]Calc. for **3**–**6** ($C_{13}H_{22}O_{11}$): 354. Calc. for **7** and **8** ($C_{12}H_{20}O_{11}$): 340.

derivatives. The observed optical rotation data, D_v values, and equivalent weights are given in Table I. These isomers were well separated from each other by ion-exchange chromatography.

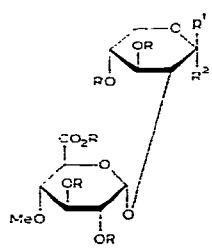
The anomeric configurations of the xylose residues of **3**–**8** were determined by ^{13}C-n.m.r. spectroscopy. The signal assignments were made on the basis of reported



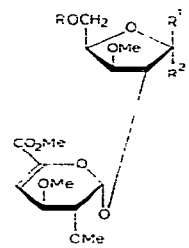
1



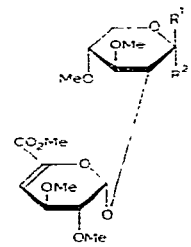
	R¹	R²	R
3	H	OMe	H
4	OMe	H	H
9	H	OMe	Me
10	OMe	H	Me



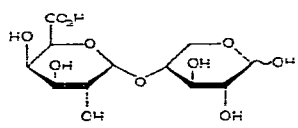
	R¹	R²	R
5	H	OMe	H
6	OMe	H	H
11	H	OMe	Me
12	OMe	H	Me



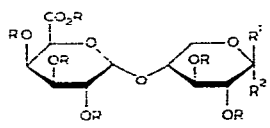
	R¹	R²
15	H	OMe
16	OMe	H



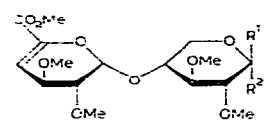
	R¹	R²
17	H	OMe
18	OMe	H



2



	R¹	R²	R
7	H	OMe	H
8	OMe	H	H
13	H	OMe	Me
14	OMe	H	Me



	R¹	R²
19	H	OMe
20	OMe	H

assignments[4-9] and comparison with chemically related compounds. Not all of the resonances were resolved. There should be 13 resonances for **3–6** and 12 for **7** and **8**, but the resolved resonances were 12 for the former and 11 for the latter.

On the basis of the broad generalisations recognised in the literature, these resonances can be subdivided into five groups, namely (*a*) the anomeric carbon atoms (97–108 p.p.m.), (*b*) the methoxyl carbon atoms (55–62 p.p.m.), (*c*) the non-anomeric, skeleton carbon atoms (in furanoid and pyranoid form) (70–85 p.p.m.), (*d*) the non-anomeric, skeleton carbon atoms substituted with methoxyl groups and glycosyl residues (76–87 p.p.m.), and (*e*) carboxyl groups (~175 p.p.m.).

For pyranoid compounds, it has been established[4,10] that (*a*) methylation of a hydroxyl group causes an 8–11-p.p.m. downfield shift in the resonance of the directly attached carbon atom; (*b*) when both the methoxyl and the adjacent hydroxyl groups are equatorial, the shift of the β-carbon atom resonance on methylation is <1 p.p.m.; (*c*) *O*-glycosylation results in a downfield shift (5–8 p.p.m.) of the signal for the substituted carbon atom; and (*d*) axially substituted, anomeric carbon atoms (α-D linkage) resonate at higher field than those equatorially substituted (β-D linkage).

On the assumption that the chemical shifts of C-1′–C-6′ of uronic acid residues of **3–8** and MeO-4′ of **3–6** are almost independent of the configuration of the methyl xyloside residues, the assignments of these resonances shown in Table II were based on those for methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid[5,6] and α-D-galacturonic acid[9]. The signals at 97.7–99.1 p.p.m. in the spectra of **3–6** and at 100.9 and 101.1 p.p.m. in the spectra of **7** and **8** were assigned to C-1′, because the uronic acid residues are linked by α linkages. The other signals in this region were assigned to C-1 of the methyl D-xyloside residues and indicated the xyloside residues of **3**, **5**, and **7** to be in the α form, and those in **4**, **6**, and **8** to be in the β form. The optical rotation data (Table I) accord with these conclusions.

The C-2–C-5 and MeO-1 resonances of **3–8** could be assigned on the basis of

TABLE II

^{13}C-N.M.R. CHEMICAL SHIFTS FOR METHYL GLYCOSIDES OF ALDOBIOURONIC ACIDS[a]

Compound	*C-1′*	*C-2′*	*C-3′*	*C-4′*	*C-5′*	*C-6′*	*MeO-4′*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*MeO-1*
	4-O-Me-α-D-GlcAp residue							D-*Xylose residue*					
3	99.1	72.1	73.0	82.8*	72.1	175.2	60.9	101.0	83.1*	74.1	78.8	61.4	56.0
4	99.1	71.7	73.0	82.6	71.7	175.2	61.0	107.8	86.6	74.4	83.4	61.8	56.2
5	97.7	72.1	73.0	83.1	72.1	175.1	60.8	97.3	76.6	72.3	70.3	61.7	55.8
6	98.6	71.9	72.9	82.6	71.9	175.1	60.8	105.2	78.5	74.9	70.3	65.7	58.2
	α-D-GalAp residue												
7	101.1	68.8	69.6	70.9	70.9	173.8		100.0	71.7	72.9	79.3	60.6	56.0
8	100.9	68.8	69.6	70.9	70.9	173.6		104.5	73.4	75.3	78.8	64.7	57.9

[a]In D_2O (δ scale); assignments marked * may be interchanged.

TABLE III

G.L.C. DATA FOR PERMETHYLATED ALDOBIOURONIC ACIDS AND THEIR 4,5-UNSATURATED HEXURONIC ACID DERIVATIVES

	Relative retention[c]											
	Aldobiouronic acid						*Unsaturated aldobiouronic acid*					
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Column *A*[a]	1.90	2.05	1.86	2.36	4.60	3.76	2.33	2.48	2.31	2.54	3.07	2.53
Column *B*[b]	1.39	1.51	1.44	1.75	3.36	2.87	1.73	1.85	1.85	2.02	2.40	2.04

[a]5–0.5% of Thermon-1000 + H_3PO_4 on Chromosorb W (80–100 mesh). [b]5% of Poly-A-101A on Chromosorb W (80–100 mesh). [c]Adjusted retention-times, relative to that of permethylated cellobiitol.

TABLE IV

E.I.- AND C.I.-MASS SPECTRA OF PERMETHYLATED ALDOBIOURONIC ACIDS **9–14**

m/z	*Relative intensity*[a]												
	9		**10**		**11**		**12**		**13**		**14**		*Ion*
	E.i.	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	
425		1		2		16		30		280		245	$[M + 1]^+$
423		6		5									$[M - 1]^+$
394		134		213	3		3		1		7	15	*ba*B_1
393		1000		1000		23		5		25		49	*ba*A_1
379	77	10	106	16									*ba*E_1
361		52		95	16	1000	6	1000		8			*ba*A_2
319	4		5		21		57		159		57		*ba*F_1
287					4		18						*ba*F_1 − 32
265						10		12		30		37	*ab*D_1
235	22	6	10	9	336	22	206	14	308	21	290	19	*ab*J_1
233	37	33	65	33	64	192	111	202	986	1000	579	1000	*a*A_1
201	1000	43	1000	184	561	447	791	447	589	15	276	14	*a*A_2
175	15		39	13	161		222	10	223		118		*b*A_1
173	32		56		20		20		90		42	7	*a*C_2
169	37		16		22	184	29	84	39		19		*a*A_3
161									39	196	17	182	
159	21		49	19	12		9		39		22		*b*C_2
157	32				18		28		8		36		
145	13	18	25	7	7		7		275	8	188		
143	15		23		31		20		175		42		*b*A_2
141	45		27		26		31		169		104		
131	9		10		25		23		22		17		
129	20		15		6	82		87	89		39	12	
117	15		6		10		9		35	15	6	15	
115	7		5		97		24		258		65		*b*C_2
101	480		513		1000		1000		1000	16	1000	22	F_1, G_1
88	109		56		458		290		703		945		H_1
75	657		815		198		116		174		609		*b*J_1

[a]Relative to an arbitrary value of 1000 for the base peak.

TABLE V

E.I.- AND C.I.-MASS SPECTRA OF PERMETHYLATED, UNSATURATED ALDOBIOURONIC ACIDS **15–20**

m/z	*Relative intensity*[a]												
	15		**16**		**17**		**18**		**19**		**20**		*Ion*
	E.i.	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	*E.i.*	*C.i.*	
393		152		51		51		30		53		18	$[M + 1]^+$
391				45									$[M - 1]^+$
362		139		132	13	100	13	84	8		15		*ba*B_1
361		1000		1000		671		603		126			*ba*A_1
347	58	13	58	13									*ba*E_1
329		534		445	19	1000	5	1000	10	451	8	982	*ba*A_2
297		39		40		85		52	17	42	10	61	*ba*A_3
287	53		60		44		160		41		164		*ba*F_1, *ba*G_1
248	108	10	72	25	763	48	770	27	536	92	362	28	*ab*H_1
235		17		11	7	44	5	27		91		35	*ab*J_1
217										201		122	
203	45	7	23	13									
201		66		75	10	36	9	46	14	1000	15	1000	*a*A_1
189		153		88		364		372					
175	26	28	44		511	7	1000	24	211	49	293	25	*b*A_1
173		30		59		74		76					
169	176	57	195	67	333	231	436	117	119	649	291	288	*a*A_2
161									10	77	9	50	
145	8	28	6	16	18	35		19	113	151	176	103	
143	80		44		323	12	486	14	123	349	240	528	*b*A_2
131	30		21		13		26						
129	11	21	21	22		17	8	16		21		21	
115	105		95		522		329		209		420		
111	6		19		47	11	44		28	231	18	293	*b*C_2
101	179		176		358		227		43		93	23	*b*F_1, *b*G_1
99	9		23		299		292		23		60		
88	37		15		179		105		351		608		H_1
85	11		33		300		56		42		84		*a*H_2'
75	1000		1000		1000		649		1000		1000		*b*J_1
71	30		37		101		24		329		376		

[a]Relative to an arbitrary value of 1000 for the base peak.

the resonances of methyl α- and β-D-xylopyranoside[7], and methyl α- and β-D-xylofuranoside[8], and the effect of *O*-glycosylation on the chemical shifts of the contiguous and neighbouring carbon atoms.

Compounds **3–8** were methylated with methylsulphinyl carbanion and methyl iodide according to the method of Hakomori[1,11]. The methylation products were reduced with lithium aluminium hydride and then hydrolysed, reduced with sodium borodeuteride, and acetylated. The g.l.c.–m.s. analysis of the alditol derivatives[12] showed that each compound gave a partially methylated xylitol acetate and a partially methylated glucitol and/or galactitol acetate. Thus the ring sizes of xyloside residues of **3–8** were consistent with those established by ^{13}C-n.m.r. spectra.

The methylation products were also analysed by g.l.c. and g.l.c.–m.s., which revealed that Hakomori methylation of **3–6** was accompanied by β-elimination, giving appreciable amounts of permethylated, unsaturated aldobiouronic acids **15–18** in addition to the permethylated products **9–12**, whereas the methylation of **7** and **8** gave only permethylated derivatives **13** and **14**. Re-methylation of **13** and **14** converted them into the permethylated, unsaturated derivatives **19** and **20**, as expected[12]. Their retention times are given in Table III.

Characteristic mass spectra (Tables IV and V), suitable for identification purposes, were obtained for all of the derivatives, although anomeric isomers gave the same fragment ions. The spectra can be interpreted readily on the basis of the fragmentation patterns for permethylated aldobiouronic acids[13] and permethylated methyl pentofuranosides[14], and the e.i.-spectrum of **11** was identical with that reported[13].

Although the fragmentation patterns of **9–12** had much in common, the characteristic differences in the e.i.-spectra for distinguishing between the furanoside (**9** and **10**) and pyranoside (**11** and **12**) derivatives were obtained by the baE_1 ions at *m/z* 379, which arise only from the former, and by the baB_1 ions at *m/z* 394 and baF_1 ions at *m/z* 319, which are formed only from the latter. The spectra of **13** and **14** gave sets of fragment ions that were almost identical with those for **11** and **12**, and contained ions at *m/z* 161 which are characteristic for (1→4)-linked disaccharides[13,15].

Ions in the low-mass range were absent from the c.i.-spectra (Table IV). The molecular weight can be easily determined from $[M + 1]^+$ ions. The base peaks varied, *e.g.*, *m/z* 393 (baA_1) for **9** and **10**, 361 (baA_2) for **11** and **12**, and 233 (aA_1) for **13** and **14**. The c.i.-spectra of **13** and **14** contained more-intense ions at *m/z* 161 than did their e.i.-spectra.

The mass spectra of the permethylated, unsaturated aldobiouronic acids **15–20** showed diagnostic ions abH_1 at *m/z* 248, which are formed by retro-Diels–Alder fragmentation of the olefinic ring[16,17]. The abH_1 ions were less pronounced in the e.i.-spectra of the furanoside derivatives **15** and **16**, compared with those of the pyranosides **17–20**, and very weak in the c.i.-spectra of all of the compounds. The aH_2' ions at *m/z* 85 were also detected in the e.i.-spectra of **15–20**; these ions are characteristic of methyl (methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosid)uronate[16].

The characteristic ions for the furanoside (**15** and **16**) and pyranoside (**17–20**) derivatives were observed in the e.i.-spectra. The spectra of **15** and **16** contained the ions baE_1 at *m/z* 347, whereas those of **17–20** contained the ions baB_1 at *m/z* 362 and baF_1 at *m/z* 287. These ions are 32 mass-units less than those of compounds **9–14**. The ions at *m/z* 287 in the e.i.-spectra of **15** and **16** are assigned to the ions baG_1 which are produced from the baE_1[14]. The baG_1 ions were absent from the spectra of **9** and **10**.

The c.i.-spectra of **15–20** showed $[M + 1]^+$ ions at *m/z* 393, and the base

peaks were the *ba*A_1 ions at *m/z* 361 for **9** and **10**, the *ba*A_2 ions at *m/z* 329 for **11** and **12**, and the *a*A_1 ions at *m/z* 201 for **13** and **14**.

Chromatography of the methylation products of **6** gave the pure β-elimination product **18** together with **12**. On the other hand, methylation of **8** gave mainly the permethylated aldobiouronic acid **14**, which, on re-methylation, yielded the permethylated, unsaturated aldobiouronic acid **20** (~31%). Compounds **18** and **20** showed an absorption at 238 nm and reacted with thiobarbituric acid, yielding a pink product (λ_{max} 550 nm), which are characteristic of a 4,5-unsaturated uronic acid residue[18,19]. The ^{1}H-n.m.r. spectra of **18** and **20** each showed a doublet at 6.14 p.p.m. assignable to H-4′ of 4,5-unsaturated 4-deoxyhexuronate[20]. On hydrolysis with 2M trifluoroacetic acid, followed by reduction with sodium borodeuteride and acetylation, **18** and **20** gave peracetylated 3,4- and 2,3-di-*O*-methyl-D-xylitol-*1-d*, respectively.

EXPERIMENTAL

General methods. — Optical rotations were measured with an automatic polarimeter (JASCO model DIP-SL). U.v. measurements were performed on a Hitachi EPS-3T spectrometer. For g.l.c., a Shimadzu GC-1C instrument, fitted with flame-ionisation detectors, was used. Separations were performed on glass columns (187.5 × 0.3 cm) containing *A*, 5–0.5% of Thermon-1000 + H_3PO_4 on Chromosorb W (80–100 mesh) at 220°; *B*, 5% of Poly-A-101 A on Chromosorb W (80–100 mesh) at 220°; and *C*, 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 180°.

E.i.- and c.i.-mass spectra were recorded on a JEOL JMS-D 100 combined gas chromatograph–mass spectrometer equipped with a chemical-ionisation source. High-resolution mass spectra were measured with a JEOL JMS-D 300 instrument interfaced with a JEOL-2000 mass-data analysis system. E.i.-mass spectra were obtained at 20-eV and 300-μA ionisation current. C.i.-mass spectra were obtained with isobutane at 0.5–1 Torr, as reagent gas. The ionising potential was 300 eV, and the ion-source temperature was maintained at 100°.

^{13}C-N.m.r.- and ^{1}H-n.m.r.-spectra were recorded on a JEOL FX 100 spectrometer, equipped with a Fourier-transform system having an 8k memory, by use of a glass tube of outside diameter 5 mm. Proton-decoupled, ^{13}C-n.m.r. spectra were recorded at 25.05 MHz, operating in the deuterio-lock mode at a probe temperature of 30°, for solutions (5–10%) of the sugars in D_2O. Chemical shifts are given relative to that of internal *p*-dioxane, which was taken as 67.4 p.p.m. downfield from tetramethylsilane.

Ion-exchange chromatography. — Acidic sugars were separated on Aminex A-27 and Diaion (AcO^-) resins by elution with *A*, 0.02M sodium acetate; *B*, 0.08M sodium acetate; *C*, 0.5M acetic acid; and *D*, M acetic acid[21]. The volume distribution coefficients (D_v) were calculated in the usual way[22].

Methylation methods. — Methylation was performed by a modification[11] of a standard method[1]. The sample (5 mg) in methyl sulphoxide (1 ml) and M sodium methylsulphinylmethanide in methyl sulphoxide (1 ml) was agitated for 2 h under

nitrogen in an ultrasonic bath (maintained at 25° by a Haake Type FE constant temperature circulator). Methyl iodide (2 ml) was then added with external cooling and the reaction mixture was agitated in an ultrasonic bath for 1 h. After distillation of excess of methyl iodide, water (3 vol.) was added and the mixture was extracted with chloroform (5 × 15 ml). The combined organic phases were washed with water (5 × 15 ml), dried with calcium chloride, and concentrated to dryness.

When a larger amount of sample (40 mg) was taken, 5 ml of methyl sulphoxide and M sodium methylsulphinylmethanide and 10 ml of methyl iodide were used.

Methyl glycosides of aldobiouronic acids (**3–8**). — 2-*O*-(4-*O*-Methyl-α-D-glucopyranosyluronic acid)-D-xylose (**1**) and 4-*O*-(α-D-galactopyranosyluronic acid)-D-xylose (**2**) were isolated from the hydrolysate of birch 4-*O*-methylglucuronoxylan with 2M trifluoroacetic acid for 2 h by ion-exchange chromatography as described previously[21,23] (D_c: **1**, 2.85 and 4.52; **2**, 3.72 and 3.75; solvents *B* and *D*, respectively). Compounds **1** and **2** (50 mg) were methanolysed with boiling, anhydrous 1.5% methanolic hydrogen chloride for 3 h. After neutralisation with silver carbonate and evaporation of methanol, the product mixtures were saponified at pH 10 and separated by ion-exchange chromatography (solvents *A* and *C*), to give four isomers (**3–6**) from **1** and two isomers (**7** and **8**) from **2**, respectively. Hydrolysis of **3–8** with 2M trifluoroacetic acid at 100° for 2 h regenerated the respective parent acids **1** or **2**, which had the same D_c values (ion-exchange chromatography with solvents *B* and *D*) as described above.

Methylation of **3–8** and reduction of the products (**9–14**) with lithium aluminium hydride in ethyl ether, in the usual way, was followed by hydrolysis with 2M trifluoroacetic acid at 100° for 2 h, reduction with sodium borodeuteride, and acetylation with pyridine–acetic anhydride. The partially methylated alditol acetates were analysed[12] by g.l.c. and g.l.c.–m.s. using column *C*. Compounds **9** and **10** gave 1,2,4-tri-*O*-acetyl-3,5-di-*O*-methyl-D-xylitol-*1-d* and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol-*1-d*. Compounds **11** and **12** gave 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-D-xylitol-*1-d* and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-glucitol-*1-d*. Compounds **13** and **14** gave 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-D-xylitol-*1-d* and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methyl-D-galactitol-*1-d*.

*Methyl 3,4-di-*O*-methyl-2-*O*-(methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronate)-β-D-xylopyranoside* (**12**) and *methyl 3,4-di-*O*-methyl-2-*O*-(methyl 4-deoxy-2,3-di-*O*-methyl-β-L-*threo*-hex-4-enopyranosyluronate)-β-D-xylopyranoside* (**18**). — The methylation products of **6** (40 mg) were chromatographed on a column (30 × 1 cm) of silica gel by elution with benzene, to give chromatographically homogeneous **12** and **18**.

Compound **12** (yield, 11.9 mg) had $[\alpha]_D^{25}$ +81° (*c* 0.6, chloroform). Mass spectrum: *m/z* 394.1794 (*ba*B_1, $C_{17}H_{30}O_{10}$, $M^+ - CH_2O$, 394.1837), 319.1440 (*ba*F_1, $C_{14}H_{23}O_8$, $M^+ - C_4H_9O_3$, 319.1391), 235.1203 (*ab*J_1, $C_{10}H_{19}O_6$, $M^+ - C_8H_{13}O_5$, 235.1180), and 233.1041 (*a*A_1, $C_{10}H_{17}O_6$, $M^+ - C_8H_{15}O_5$, 233.1024).

Compound **18** (yield, 8.3 mg) had $[\alpha]_D^{25}$ +135° (*c* 0.4, chloroform), λ_{max}^{MeOH} 238 nm (ε 5600). Reaction of **18** with thiobarbituric acid yielded a pink product

(λ_{max} 550 nm). Hydrolysis of **18** with 2M trifluoroacetic acid at 120° for 1 h, followed by reduction with sodium borodeuteride and acetylation, gave 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-D-xylitol-*1-d*. ^{1}H-N.m.r. data: δ 6.14 (d, 1 H, $J_{3',4'}$ 2.7 Hz, H-4'), 5.68 (d, 1 H, $J_{1',2'}$ 2.4 Hz, H-1'), 4.26 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.10 (dd, 1 H, $J_{2',3'}$ 7.5, $J_{3',4'}$ 2.7 Hz, H-3'), 3.80 (s, 3 H, CO_2Me), 3.53–3.37 (5 s, 15 H, 5 OMe), and 2.94–3.10 (m, 2 H, H-5,5); the signals of other protons were superposed on those of the methoxyl groups. Mass spectrum: *m/z* 362.1571 (*ba*B_1, $C_{16}H_{26}O_9$, $M^+ - CH_2O$, 362.1575), 329.1222 (*ba*A_2, $C_{15}H_{21}O_8$, $M^+ - C_2H_7O_2$, 329.1235), 287.1110 (*ba*F_1, $C_{13}H_{19}O_7$, $M^+ - C_4H_9O_3$, 287.1130), 248.1244 (*ab*H_1, $C_{11}H_{20}O_6$, $M^+ - C_6H_8O_4$, 248.1259), and 201.0746 (*a*A_1, $C_9H_{13}O_5$, $M^+ - C_8H_{15}O_5$, 201.0762).

*Methyl 2,3-di-*O*-methyl-4-*O*-(methyl 2,3,4-tri-*O*-methyl-α-*D*-galactopyranosyluronate)-β-*D*-xylopyranoside* (**14**). — The methylation products of **8** (40 mg) were chromatographed on a column (40 × 1 cm) of silica gel by elution with benzene–acetone (10:1), to give chromatographically pure **14** (26.7 mg), $[\alpha]_D^{25}$ +66° (*c* 1.3, chloroform). Mass spectrum: *m/z* 394.1855 (*ba*B_1, $C_{17}H_{30}O_{10}$, $M^+ - CH_2O$, 394.1837), 319.1412 (*ba*F_1, $C_{14}H_{23}O_8$, $M^+ - C_4H_9O_3$, 319.1391), 235.1220 (*ab*J_1, $C_{10}H_{19}O_6$, $M^+ - C_8H_{13}O_5$, 235.1180), and 233.1034 (*a*A_1, $C_{10}H_{17}O_6$, $M^+ - C_8H_{15}O_5$, 233.1024).

*Methyl 2,3-di-*O*-methyl-4-*O*-(methyl 4-deoxy-2,3-di-*O*-methyl-β-*L*-threo-hex-4-enopyranosyluronate)-β-*D*-xylopyranoside* (**20**). — Compound **14** (25 mg) was remethylated and the products were chromatographed on a column (40 × 1 cm) of silica gel by elution with benzene–acetone (10:1), to give chromatographically pure **20** (7.8 mg), $[\alpha]_D^{25}$ +107° (*c* 0.4, chloroform), λ_{max}^{MeOH} 238 nm (ε 6500). Reaction of **20** with thiobarbituric acid yielded a pink product (λ_{max} 550 nm). Hydrolysis of **20** with 2M trifluoroacetic acid at 120° for 1 h, followed by reduction with sodium borodeuteride and acetylation, gave 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-D-xylitol-*1-d*. ^{1}H-N.m.r. data: δ 6.14 (d, 1 H, $J_{3',4'}$ 2.7 Hz, H-4'), 5.41 (d, 1 H, $J_{1',2'}$ 2.7 Hz, H-1'), 4.08 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.04 (dd, 1 H, $J_{2',3'}$ 2.7, $J_{3',4'}$ 7.5 Hz, H-3'), 3.80 (s, 3 H, CO_2Me), 3.63–3.56 (5 s, 15 H, 5 OMe), and 2.84–3.24 (m, 2 H, H-5,5); the signals of other protons were superposed on those of the methoxyl groups. Mass spectrum: *m/z* 362.1572 (*ba*B_1, $C_{16}H_{26}O_9$, $M^+ - CH_2O$, 362.1575), 329.1252 (*ba*A_2, $C_{15}H_{21}O_8$, $M^+ - C_2H_7O_2$, 329.1235), 287.1130 (*ba*F_1, $C_{13}H_{19}O_7$, $M^+ - C_4H_9O_3$, 287.1130), 248.1284 (*ab*H_1, $C_{11}H_{20}O_6$, $M^+ - C_6H_8O_4$, 248.1259), and 201.0800 (*a*A_1, $C_9H_{13}O_5$, $M^+ - C_8H_{15}O_5$, 201.0762).

REFERENCES

1 S. Hakomori, *J. Biochem. (Tokyo)*, 55 (1964) 205–208.
2 K. Shimizu, *Mokuzai Gakkaishi*, 21 (1975) 662–668.
3 K. Shimizu, *Mokuzai Gakkaishi*, 22 (1976) 51–53.
4 D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, 92 (1970) 1355–1361.
5 J. Y. Lee, A. Ishizu, S. Hosoya, and J. Nakano, *Cellulose Chem. Technol.*, 13 (1979) 739–745.
6 A. S. Shashkov, A. F. Sviridov, O. S. Chizhov, and P. Kováč, *Carbohydr. Res.*, 62 (1978) 11–17.
7 P. A. J. Gorin and M. Mazurek, *Carbohydr. Res.*, 48 (1976) 171–186.
8 P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 53 (1975) 1212–1223.

9 K. IZUMI, *Agric. Biol. Chem.*, 44 (1980) 1623–1631.
10 P. COLSON, H. J. JENNINGS, AND I. C. P. SMITH, *J. Am. Chem. Soc.*, 96 (1974) 8081–8087.
11 P. J. GAREGG, B. LINDBERG, T. ONN, AND T. HOLM, *Acta Chem. Scand.*, 25 (1971) 1185–1194.
12 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem. Int. Ed. Engl.*, 9 (1970) 610–619.
13 V. KOVÁČIK, Š. BAUER, J. ROSIK, AND P. KOVÁČ, *Carbohydr. Res.*, 8 (1968) 282–290.
14 V. KOVÁČIK AND P. KOVÁČ, *Carbohydr. Res.*, 24 (1972) 23–28.
15 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.
16 V. KOVÁČIK AND P. KOVÁČ, *Anal. Biochem.*, 64 (1975) 45–52.
17 J. HIRSH, P. KOVÁČ, AND V. KOVÁČIK, *Carbohydr. Res.*, 56 (1977) 391–397.
18 P. ALBERSHEIM, H. NEUKOM, AND H. DEUEL, *Arch. Biochem. Biophys.*, 90 (1960) 46–51.
19 J. KISS, *Adv. Carbohydr. Chem. Biochem.*, 29 (1974) 230–298.
20 J. ALFÖLDI, R. PALOVČÍK, C. PECIAR, J. HIRSCH, AND P. KOVÁČ, *Carbohydr. Res.*, 44 (1975) 133–137.
21 K. SHIMIZU, M. HASHI, AND K. SAKURAI, *Carbohydr. Res.*, 62 (1978) 117–126.
22 O. SAMUELSON, *Ion Exchange Separations in Analytical Chemistry*, Almqvist and Wiksell, Stockholm; Wiley, New York; 1963, pp. 125–129.
23 K. SHIMIZU, M. ISHIHARA, AND T. ISHIHARA, *Mokuzai Gakkaishi*, 22 (1976) 618–625.